植 物 分 类 学 报 44 (5): 516–522(2006) doi:10.1360/aps050030
Acta Phytotaxonomica Sinica http://www.plantsystematics.com

Chromosome numbers of four genera in the Dryopteridaceae

[1,2]LU Jin-Mei [1]LI De-Zhu* [1]WU Ding

[1] (*Laboratory for Plant Biodiversity and Biogeography, Kunming Institute of Botany, the Chinese Academy of Sciences*, Kunming 650204, China)

[2] (*Graduate School of the Chinese Academy of Sciences*, Beijing 100039, China)

Abstract Chromosome numbers and reproductive types of six species of four genera in the Dryopteridaceae, i.e., *Leptorumohra*, *Lithostegia*, *Phanerophlebiopsis* and *Cyrtogonellum* were investigated. The chromosome numbers were *n*=41 in *Leptorumohra quadripinnata*, 2*n*=164 in *Lithostegia foeniculacea*, 2*n*=82 in *Phanerophlebiopsis neopodophylla*, '*n*'=123, and 2*n*=123 in *Cyrtogonellum fraxinellum*, '*n*'=123 in *C. caducum* and 2*n*=123 in *C. inaequalis*, respectively. The chromosome numbers for all the genera but *Leptorumohra* were reported for the first time. The results indicated that the chromosome base number of all the four genera was *x*=41, a common number in the Dryopteridaceae. The reproductive type in *Cyrtogonellum* is apogamous whereas in the other three genera it is of the sexual reproductive type, which is widely found in ferns.

Key words Dryopteridaceae, *Cyrtogonellum*, *Leptorumohra*, *Lithostegia*, *Phanerophlebiopsis*, chromosome number, apogamy.

The family Dryopteridaceae is of cosmopolitan distribution although with a strong concentration of genera and species in the temperate regions of the Northern Hemisphere, particularly in the hills and mountains of eastern Asia (Ching, 1965a; Tryon & Tryon, 1982; Kramer, 1990; Wu & Ching, 1991; Wu, 2000). Ching (1965a) founded the family Dryopteridaceae based on *Dryopteris* Adans. However, the Dryopteridaceae has been variously circumscribed ever since. The family was treated under an illegitimate name, Aspidiaceae, by many authors (e.g. Pichi-Sermolli, 1977), and it was more broadly defined to include Athyriaceae, Woodsiaceae, Lomariopsidaceae, Nephrolepidaceae, Onocleaceae, and Tectariaceae (Tryon & Tryon, 1982; Tryon & Lugardon, 1990; Kramer, 1990). However, recent molecular phylogenetic studies using *rbc*L sequence data revealed the polyphyly of the Dryopteridaceae sensu Kramer (1990) (Hasebe et al., 1994, 1995; Wolf et al., 1994; Pryer et al., 1995; Sano et al., 2000), although phylogenetic relationships of genera in Dryopteridaceae were not well supported. Furthermore, there is no cladistic analysis of morphologic and cytotaxonomic data, which made the phylogenetic relationships within the family more unresolved. Any sound subdivision of it could be very provisional without further studies on morphological, cytological and molecular data.

Cytological and reproductive studies may provide very useful information for the phylogenetic studies of various groups of organisms (Raven, 1975). Chromosome counts can promote our understanding of phylogenetic relationships at different taxonomic levels. Some cytological observations of the family Dryopteridaceae have been reported since 1950s (Manton, 1950; Mitui, 1965; Hirabayashi, 1969; Tsai & Shieh, 1985; Lovis, 1977; Löve et al., 1977; Shimura et al., 1982; Wang & Xia, 1984; Wang, 1985; Weng, 1990; Kato et al., 1992;

Received: 23 February 2005 Accepted: 1 July 2005
Supported by the Yunnan Provincial Government through an Award for Prominent Contributions in Science and Technology to Prof. Wu Zheng-Yi in 2001, No. KIB-WU-2001-03.
* Author for correspondence. E-mail: <dzl@mail.kib.ac.cn>.

Yatskievych, 1996; Lu & Cheng, 2003). Chromosome counts published previously for genera of the family indicated that the chromosome base number for these genera was x=41. However, there is still a blank of chromosome counts in some genera, especially in those from eastern Asia. One goal of this study is to increase the sampling size of the genera for chromosome counts in the Dryopteridaceae, as part of our phylogenetic analyses combining morphological, cytological and molecular evidence. For practical reason, the classification of the Dryopteridaceae in the *Flora Reipublicae Popularis Sinicae* (Wu, 2000; Kung, 2001) was followed herein.

1 Material and methods

Living plants (Table 1) were collected in the field in Yunnan Province and Chongqing Municipality, China, and cultivated in the Kunming Botanical Garden, Kunming Institute of Botany, the Chinese Academy of Sciences. Voucher specimens are deposited in the Herbarium of Kunming Institute of Botany, the Chinese Academy of Sciences (KUN).

Table 1 Materials and chromosome counts of four genera of Dryopteridaceae

Taxon	Chromosome number	Spore Number[1)]	Locality	Voucher
Leptorumohra quadripinnata (Hayata) H. Ito	n = 41	64	Dali, Yunnan (云南大理)	J. M. Lu et al. (卢金梅等) 112 (KUN)
Lithostegia foeniculacea (Hook.) Ching	2n = 164	64	Gongshan, Yunnan (云南贡山)	J. M. Lu et al. (卢金梅等) 155 (KUN)
Phanerophlebiopsis neopodophylla (Ching) Ching ex Y. T. Xie	2n = 82	64	Guangnan, Yunnan (云南广南)	J. M. Lu et al. (卢金梅等) 176 (KUN)
Cyrtogonellum fraxinellum (Christ) Ching	'n' = 123, 2n = 123	32	Xichou, Yunnan (云南西畴)	J. M. Lu et al. (卢金梅等) 002 (KUN)
Cyrtogonellum caducum Ching	'n' = 123	32	Xichou, Yunnan (云南西畴)	J. M. Lu et al. (卢金梅等) 001 (KUN)
Cyrtogonellum inaequalis Ching	2n = 123	32	Mt. Jinfoshan, Chongqing (重庆金佛山)	J. M. Lu et al. (卢金梅等) 047 (KUN)

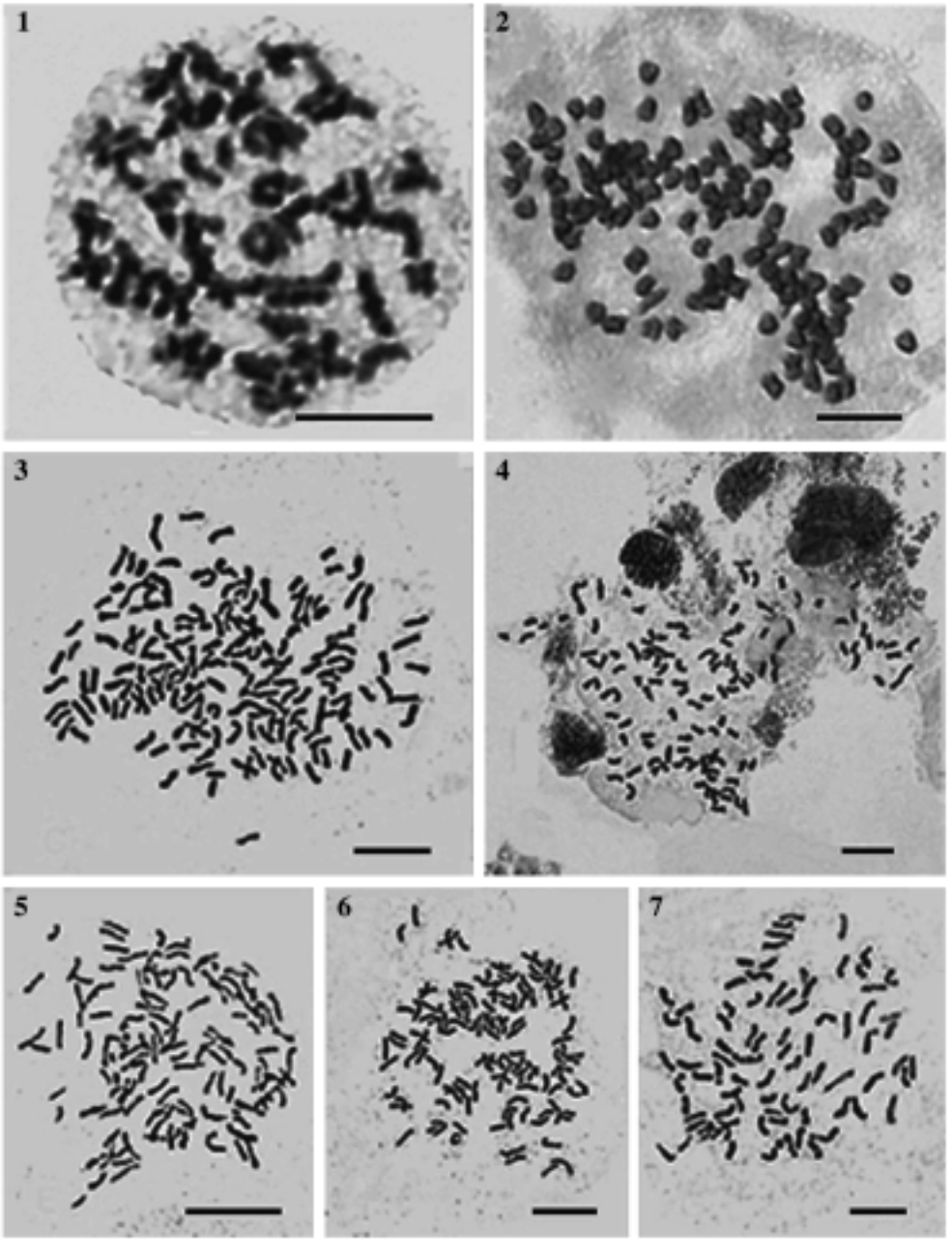
[1)] Number of spores per sporangium.

Root tips were treated in 0.002 mol/L 8-hydroxyquinoline solution for 3–4 h before being fixed in Carnoy's solution (95% ethanol : ice acetic acid=3:1) for 4 h. They were washed in 95%, 70%, 50% ethanol and distilled water, and hydrolyzed in 1 mol/L HCl at 60 ℃ for 9–12 min, and then stained in 2% aceto-orcein for 1–3 h and squashed in 45% acetic acid. Meiotic chromosome counts were gained from sporocytes at late diplotene or diakinesis. The suitable pinna (on which the sori slightly became yellow) was fixed in Carnoy's solution using the usual aceto-carmine squash method (Manton, 1950). Mitotic and meiotic cells were examined and photographed using a Zeiss Axiophot-II photomicroscope.

To determine the reproductive type, number of spores per sporangium was counted. According to Walker (1979), there were two reproductive types in ferns, i.e., the sexual reproductive type with 64 spores in a sporangium, and the apogamous one with 32 spores.

2 Results

The chromosome photographs of meiotic and/or mitotic materials of six species in four genera are shown in Figs. 1–7. These chromosome counts (Table 1) are all reported for the first time.



Figs. 1–7. Chromosomes of four genera of Dryopteridaceae at meiosis and mitosis. **1.** *Leptorumohra quadripinnata*, *n*=41. **2.** *Cyrtogonellum caducum*, '*n*' = 123. **3.** *Lithostegia foeniculacea*, 2*n*=164. **4.** *Cyrtogonellum fraxinellum*, '*n*'=123. **5.** *Cyrtogonellum inaequalis*, 2*n*=123. **6.** *Cyrtogonellum fraxinellum*, 2*n*=123. **7.** *Phanerophlebiopsis neopodophylla*, 2*n*=82. Scale bars=10 μm.

2.1 ***Leptorumohra quadripinnata*** **(Hayata) H. Ito**

The species is distributed in Yunnan, Guangxi and Taiwan, China and also in Japan. The material from Dali, NW Yunnan, had 41 bivalents at meiosis (Fig. 1). Sixty-four regularly-shaped spores were observed per sporangium. Thus this species is a sexual diploid.

2.2 ***Lithostegia foeniculacea*** **(Hook.) Ching**

This is a Himalayan species, distributed in India, Sikkim, Myanmar and southwestern China (Xizang and Yunnan). The material from Gongshan, NW Yunnan, was proved to be a sexual tetraploid having a somatic chromosome number of 2*n*=164 (Fig. 3), and producing 64 spores per sporangium.

2.3 ***Phanerophlebiopsis neopodophylla*** **(Ching) Ching ex Y. T. Xie**

This species is endemic to China, occurring in Guizhou and Yunnan. The material from Guangnan, SE Yunnan, had 82 chromosomes in the mitotic cells (Fig. 7). The spores are regular in shape. The species is a sexual diploid, with 64 spores in a sporangium.

2.4 ***Cyrtogonellum fraxinellum*** **(Christ) Ching**

This species is distributed in Yunnan, Guizhou, Guangxi, and Taiwan, China. The material from Xichou, SE Yunnan, was proved to be of apogamous reproductive type, with the same chromosome number of 123 both in meiosis and mitosis (Figs. 4, 6), and with 32 spores in a sporangium. Thus this species is an apogamous triploid.

2.5 ***Cyrtogonellum caducum*** **Ching**

This species is distributed in Chongqing, Guangxi, Guizhou, Sichuan, and Yunnan, China. The material from Xichou, SE Yunnan, had 123 bivalents at meiosis (Fig. 2). Thirty-two regular spores were observed in a sporangium. Thus this species is an apogamous triploid.

2.6 ***Cyrtogonellum inaequalis*** **Ching**

This species is distributed in Chongqing and Guizhou, China. The material from Mt. Jinfoshan, Chongqing, had 123 chromosomes in the mitotic cells of root tips (Fig. 5). Thirty-two regular spores were observed in each sporangium. Thus this species is an apogamous triploid.

3 Discussion

The chromosome numbers for the genera *Lithostegia*, *Phanerophlebiopsis* and *Cyrtogonellum* are reported for the first time. These results indicate that the chromosome base number of all the three genera is *x*=41, a common number in the Dryopteridaceae. The fourth genus, *Leptorumohra*, is an eastern Asian genus of four species. *Leptorumohra miqueliana* (Maxim. ex Franch. & Sav.) H. Ito was cytologically studied by Hirabayashi in 1969. He reported there were intraspecific polyploids in this species in Japan, including diploid, triploid and tetraploid. From the result of the present observation, *L. quadripinnata* is a sexual diploid. However, polyploidy may be found in this species after observing more populations.

Lithostegia is a monotypic genus based on *Aspidium foeniculaceum* Hook. (Ching, 1933). The particular morphology of indusium makes *Lithostegia* very distinct in Dryopteridaceae. The phylogenetic relationship of *Lithostegia* is not in consensus (Nayar & Kaur, 1963; Pichi-Sermolli, 1977; Kramer, 1990). The base number of *x*=41 as revealed here provides cytological evidence in support of its placement in Dryopteridaceae. *Phanerophlebiopsis* is a genus of nine species endemic to China, mainly distributed in Guizhou, Hunan and their neighbouring regions (Ching, 1965b, 1987; Xie, 1990b). The present study has revealed that this genus has *x*=41, strongly suggesting that it should be a member of the Dryopteridaceae.

There are two different reproductive types, the sexual type and the apogamous one, in

ferns (Manton, 1950). About 10% of the world's (Lovis, 1977) and about 13% Chinese (Kato & Nakato, 1999) fern species are apogamous. The reproductive type in *Cyrtogonellum* is apogamous while in the above-mentioned three genera it is sexual, a reproductive type widely found in ferns. *Cyrtogonellum* is a genus distributed in limestone areas in southwestern China to northern Vietnam, comprising about eight species (Xie & Li, 1989; Xie, 1990a). Apogamous ferns are mostly triploid (Lovis, 1977; Walker, 1979). From our previous (Lu & Cheng, 2003) and present observations, it seems that there is a similar tendency of apogamy in conjunction with triploidy in *Cyrtomium* Presl and *Cyrtogonellum*. All the three examined *Cyrtogonellum* species and eight apogamous *Cyrtomium* species are triploid (Lu & Cheng, 2003; Lu et al., 2006). These species are all distributed in limestone areas with relatively dry condition and infertile soil. In such a habitat, seasonal shortage of water may significantly affect the reproductive processes in ferns. If water availability is low, fern sperms cannot move freely to achieve fertilization successfully. However, apogamy can avoid this shortcoming, as it does not need water for fertilization. This may explain that apogamous ferns can occur in drier and colder habitats than those with only sexual reproductive type. Apogamy may have evolved in *Cyrtomium* and *Cyrtogonellum* as a means of adaptation to degraded habitats during their evolutionary processes.

Acknowledgements We would like to thank Prof. LU Shu-Gang of the Yunnan University, to Dr. GAO Lian-Ming and Ms. JIAO Yu of Kunming Institute of Botany, the Chinese Academy of Sciences for their help in collecting materials, to Prof. WANG Hong and Prof. GU Zhi-Jian of Kunming Institute of Botany, the Chinese Academy of Sciences for their kind help in experiments.

References

Ching R-C. 1933. *Lithostegia*, a new genus of Polypodiaceous fern from Sikkim-Yunnan. Sinensia 4: 1–9.

Ching R-C (秦仁昌). 1938. A revision of the Chinese and Sikkim-Himalayan *Dryopteris* with reference to some species from neighbouring regions. Bulletin of the Fan Memorial Institute of Biology 8: 275–334.

Ching R-C (秦仁昌). 1965a. Dryopteridaceae—A new fern family. Acta Phytotaxonomica Sinica (植物分类学报) 10: 1–5.

Ching R-C (秦仁昌). 1965b. Two new fern genera from China. Acta Phytotaxonomica Sinica (植物分类学报) 10: 115–122.

Ching R-C (秦仁昌). 1987. Some addition and corrections to the fern genus *Phanerophlebiopsis* Ching. Bulletin of Botanical Research (植物研究) 7: 71–75.

Hasebe M, Omori T, Nakazawa M, Sano T, Kato M, Iwatsuki K. 1994. *rbc*L gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns. Proceedings of the National Academy of Sciences, USA 91: 5730–5734.

Hasebe M, Wolf P G, Pryer K M, Ueda K, Ito M, Sano R, Gastony G J, Yokoyama J, Manhart J R, Murakami N, Crane E H, Haufler C H, Hauk W D. 1995. Fern phylogeny based on *rbc*L nucleotide sequences. American Fern Journal 85: 134–181.

Hirabayashi H. 1969. Intraspecific polyploidy in *Leptorumohra miqueliana*. The Journal of Japanese Botany 44: 185–192.

Kato M, Nakato N. 1999. A cytotaxonomic study of Hainan (S. China) pteridophytes with notes on polyploidy and apogamy of Chinese species. In: Zhang X-C, Shing K-H eds. Ching Memorial Volume. Beijing: China Forestry Publishing House. 1–19.

Kato M, Nakato N, Cheng X, Iwatsuki K. 1992. Cytotaxonomic study of ferns of Yunnan, southwestern China. The Botanical Magazine (Tokyo) 105: 105–124.

Kramer K U. 1990. Dryopteridaceae. In: Kramer K U, Green P S eds. The Families and Genera of Vascular Plants. Vol. 1. Pteridophytes and Gymnosperms. Berlin: Springer-Verlag. 101–144.

Kung H-S (孔宪需). 2001. Dryopteridaceae (2). In: Flora Reipublicae Popularis Sinicae (中国植物志). Beijing: Science Press. 5 (2): 1–228.

Löve A, Löve D, Pichi-Sermolli R E G. 1977. Cytotaxonomical Atlas of the Pteridophyta. Vaduz: A. R.

Gantner Verlag.
Lovis J D. 1977. Evolutionary patterns and processes in ferns. Advances in Botanical Research 4: 229–415.
Lu J-M (卢金梅), Cheng X (成晓). 2003. Chromosome numbers of 10 species of *Cyrtomium* (Dryopteridaceae). Acta Botanica Yunnanica (云南植物研究) 25: 663–670.
Lu J-M, Cheng X, Wu D, Li D-Z. 2006. Chromosome study of the fern genus *Cyrtomium* (Dryopteridaceae). Botanical Journal of the Linnean Society 150: 221–228.
Manton I. 1950. Problems of Cytology and Evolution in the Pteridophyta. Cambridge: Cambridge University Press.
Mitui K. 1965. Chromosome studies on Japanese ferns (1). The Journal of Japanese Botany 40: 117–124.
Nayar B K, Kaur S. 1963. Ferns of India, 9. *Peranema* and *Acrophorus*. Bulletin of the National Botanic Gardens Lucknow 81: 1–40.
Pichi-Sermolli R E G. 1977. Tentamen Pteridophytorum genera in taxonomicum ordinem redigendi. Webbia 31: 313–512.
Pryer K M, Smith A R, Skog J E. 1995. Phylogenetic relationships of extant ferns based on evidence from morphology and *rbc*L sequences. American Fern Journal 85: 205–282.
Raven P H. 1975. The bases of angiosperm phylogeny: cytology. Annals of the Missouri Botanical Garden 62: 724–764.
Sano R, Takamiya M, Ito M, Kurita S, Hasebe M. 2000. Phylogeny of the lady fern group, tribe Physematieae (Dryopteridaceae) based on chloroplast *rbc*L gene sequences. Molecular Phylogenetics and Evolution 15: 403–413.
Shimura Y, Matsumoto S, Tsuzimura A, Sugiyama M. 1982. A study of the chromosome numbers of some taxa of Japanese *Arachniodes*. The Journal of Phytogeography and Taxonomy 30: 19–26.
Tryon A F, Lugardon B. 1990. Spores of the Pteridophyta: Surface, Wall Structure, and Diversity Based on Electron Microscope Studies. New York: Springer-Verlag.
Tryon M R, Tryon A F. 1982. Ferns and Allied Plants (With Special Reference to Tropical America). New York: Springer-Verlag. 454–542.
Tsai J-L, Shieh W-C. 1985. A cytotaxonomic survey of the fern family Aspidiaceae (sensu Copland) in Taiwan. Journal of Science and Engineering, NCHU 22: 121–144.
Walker T G. 1979. The Cytogenetics of Ferns. In: Dyer A F ed. The Experimental Biology of Ferns. London: Academic Press. 87–132.
Wang Z-R (王中仁). 1985. Three new cytotypes of *Dryopteris chrysocoma* complex. Acta Phytotaxonomica Sinica (植物分类学报) 23: 344–351.
Wang Z-R (王中仁), Xia Q (夏群). 1984. Cytological observations on some species of *Dryopteris* and *Polystichum* from China. Acta Botanica Sinica (植物学报) 26: 24–27.
Weng R-F (翁若芬). 1990. Cytological observations of some Chinese ferns. Acta Phytotaxonomica Sinica (植物分类学报) 28: 27–33.
Wolf P G, Soltis P S, Soltis D E. 1994. Phylogenetic relationships of dennstaedtioid fern: evidence from *rbc*L sequences. Molecular Phylogenetics and Evolution 3: 383–392.
Wu S-G. 2000. Dryopteridaceae (1). In: Flora Reipublicae Popularis Sinicae (中国植物志). Beijing: Science Press.
Wu S-H (吴兆洪), Ching R-C (秦仁昌). 1991. Fern Families and Genera of China (中国蕨类植物科属志). Beijing: Science Press.
Xie Y-T (谢寅堂). 1990a. Classification and distribution of *Cyrtogonellum* Ching. Bulletin of Botanical Research (植物研究) 10: 93–97.
Xie Y-T (谢寅堂). 1990b. Classification and distribution of *Phanerophlebiopsis* Ching. Bulletin of Botanical Research (植物研究) 10: 87–91.
Xie Y-T (谢寅堂), Li N (李娜). 1989. New taxa of genus *Cyrtogonellum* Ching. Bulletin of Botanical Research (植物研究) 9: 15–19.
Yatskievych G. 1996. A revision of the fern genus *Phanerophlebia* (Dryopteridaceae). Annals of the Missouri Botanical Garden 83: 168–199.

鳞毛蕨科四个属的染色体数目

[1,2]卢金梅 [1]李德铢* [1]吴 丁

[1] (中国科学院昆明植物研究所生物多样性与生物地理学重点实验室 昆明 650204)

[2] (中国科学院研究生院 北京 100039)

摘要 报道了鳞毛蕨科的4个属(毛枝蕨属*Leptorumohra*、石盖蕨属*Lithostegia*、黔蕨属*Phanerophlebiopsis*和柳叶蕨属*Cyrtogonellum*)6种植物的染色体数目及生殖方式。其中柳叶蕨*Cyrtogonellum fraxinellum* ‘*n*’=123, 2*n*=123; 离脉柳叶蕨*C. caducum* ‘*n*’=123; 斜基柳叶蕨*C. inaequalis* 2*n*=123; 四回毛枝蕨*Leptorumohra quadripinnata* *n*=41; 石盖蕨*Lithostegia foeniculacea* 2*n*=164; 长叶黔蕨*Phanerophlebiopsis neopodophylla* 2*n*=82。石盖蕨属、黔蕨属和柳叶蕨属的染色体数目为首次报道。结果表明这3个属的染色体基数和鳞毛蕨科中其他属一样均为*x*=41。细胞学证据支持将石盖蕨属、黔蕨属置于鳞毛蕨科的处理。本文还发现柳叶蕨属与贯众属*Cyrtomium*的一些种一样, 具有无融合生殖方式, 而其他3个属仅具有在蕨类植物中较为常见的有性生殖方式。

关键词 鳞毛蕨科; 柳叶蕨属; 毛枝蕨属; 石盖蕨属; 黔蕨属; 染色体数目; 无融合生殖